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Bacteriophage: A Boon for Treatment of Systemic Infections

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ABSTRACT:
Although the discovery and use of antibiotics has greatly aided in reaching new heights in healthcare, helping to effectively cure many previously incurable diseases, the rapid resistance against these antibiotics developed by bacteria is threatening to reverse this same progress. Antimicrobial Resistance or AMR is considered to be potent global emergency. Several alternatives to antibiotics are being constantly studied in the past few years, to tackle this issue of universal importance. Bacteriophage or phage as a macromolecule to help fight infections has been under the eye as a potential replacement to antibiotics for years, with increased emphasis in past decade. Phage can be easily isolated from natural sources and purified for use, making it more economical than other alternatives. It's widespread application in different types of infections, versatile drug delivery systems and subsequent success of the treatment has warranted a promising future. Phage treatment in India has gathered traction in the past few years making it an exciting prospect for the future.

Keywords: Antibiotics, Antimicrobial Resistance, Bacteriophage

I. INTRODUCTION [1]

Broad spectrum antibiotics used against wide bacteria resulted in undue misuse and overuse of those antibiotics leading to antibiotic resistance. Thus the resistance to antibiotics is a severe problem for treatment of a various infections where the bacteria becomes highly immune to variety of antibiotic products including the third generation Cephalosporin, Carbapenem and Fluoroquinolones.

II. BACTERIOPHAGE [2]

Bacteriophage or simply phage is a bacteria infecting virus. Bacteriophage was discovered independently by Fredrick W. Twort in England in 1915 and also by Felix d'Herelle at the Pasteur Institute in Paris in 1917.

The bacterial hosts of phage are easily cultivated under controlled conditions, demanding relatively little in terms of time, labour, and space compared with the upkeep of plant and animal hosts. Bacteriophage has received considerable attention in viral research. Since bacteriophage is the simplest biological entity known capable of self-replication, its need been widely utilized in genetic research. Bacteriophage usually infects their bacterial hosts in a very species- or perhaps strain-specific manner.

2.1 Characteristics of a bacteriophage:

Bacteriophage consists of a macromolecule core surrounded by a protein coat. Bacterial viruses occur in several shapes, although many have a tail through which they inoculate the host cell with viral nucleic acids. Phage is often divided into virulent and temperate phage supported their life cycle.

a. Virulent phage: responsible for the lytic cycle in which the phage attaches to the bacterial host, injects its the genome and start reproducing by using the host's molecular machinery and further cause lyses of the host cell. Thus they release the progeny. The lytic phage uses proteins for destroying the host cells. The holin perforates the bacterial cytoplasmic membrane and works as a synergy tool for the endolysins. The endolysins are therefore liable for destruction of bacterial cytomembrane.

2. Temperate phage: It initiates lysogenic cycle that affects the host cell. The phage genome remains dormant as a prophage, then replicates together with its host and rarely bursts in a lytic cycle. Lysogeny and prophage are often beneficial to the bacteria as they help in encoding the genes for antibiotic resistance or other virulence factors.

2.2 Morphology and Structure:

All phage have a macromolecule core covered by a protein coat or capsid. The capsid is created from morphological subunits called capsomers. The capsomer consists of variety of protein subunits or molecules called protomers.

Bacterial virus is also grouped into six morphological types:

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Type A: It is the foremost complex type and features a hexagonal head, a rigid tail with a contractile sheath and tail fibres.
Type B: It is just like blood group. Blood type features a hexagonal head. However it lacks a contractile sheath. It is a versatile tail and it should or might not have tail fibers.
Type C: It is characterized by a hexagonal head and a tail shorter than the top. The tail has no contractile sheath and tail fibers.
Type D: It features a head made from large capsomers but has no tail.
Type E: It features a head made from small capsomeres but has no tail.
Type F: It is filamentous.
Type A, Type B and Type C show morphology unique to bacteriophage. The morphological types in Type D and Type E are found in plant and animal viruses also. The filamentous Type F is found in some plant viruses.

III. PHAGE THERAPY [3]

Bacteriophage acts as promising viable alternative to conventional antimicrobial therapy for the treatment of bacterial infections. The bacteriophage treatment is highly specific. The bacteriophage shows replication on its own in the host and stops its action when the host is no longer prevalent. Bacteriophage kills the bacteria by bursting or lysis of the bacterial cells by binding to the bacteria. The phage reproduces within the bacteria thus making up to 1000 new viruses within the bacterial cell. Eventually the virus breaks open the bacterial cell giving out new bacteriophages. Bacteriophage can multiply and grow inside a bacterial cell. After the lysis of the bacteria, they stop multiplying.

3.1 NEED FOR PHAGE THERAPY [4, 5]

Genes that are antibiotic resistant and encode for bacterial resistance are a major concern to the medical treatment of common diseases thus many antibiotics have lesssened efficacy against common infections.
On September 21, 2016 the United Nations General Assembly convened to discuss the problem of antibiotic resistance and deemed it “the greatest and most urgent global risk”. In the hunt for alternative strategies for prophylaxis and control of bacterial infection, one of the more popular suggestions involves revisiting the practice of phage therapy. Proponents of phage therapy tout several major advantages that phage have over antibiotics such as host-specificity, self-amplification, biofilm degradation and low toxicity to humans. Owing to the development of analytical tools capable of studying these small biological entities (approximately 25-200 nm in length), such as next-generation sequencing and electron microscopy, the field of phage biology is only now reaching maturity.

3.2 MECHANISM OF ACTION OF A BACTERIOPHAGE [2,4]

Replication of bacteriophage:
Step 1 - ADSORPTION: The tip of the virus tail becomes attached to the cell via specific receptor sites on the cell surface. Attachment is particular, therein certain viruses and susceptible bacteria have complementary molecular configuration at their opposing receptor sites. In some cases the precise receptor of the bacterium is a component of the bacterial lipopolysaccharide although any surface structure can function as specific phage receptors.
Step 2 - PENETRATION: If too many phage attach to the bacterium and penetrate it, there could also be premature lysis which is not in the middle of the assembly of recent viruses. The particular penetration of phage into the host cell is mechanical. But is facilitated by localised digestion of certain cell surface structures either by phage enzymes carried on the tail of phage or by viral activation of host degradative enzyme.
In the T-even phage penetration is achieved when:

- the tail fibres of the virus attach to the cell and hold the tail firmly against the plasma membrane,
- the sheath contracts driving the tail core into the cell through the plasma membrane and membrane and
- the virus injects its DNA the way a syringe injects a vaccine.

Step 3 - TRANSCRIPTION: Bacterial mRNA and bacterial proteins stop being synthesized within some minutes after entry of phage DNA. Bacterial DNA is quickly degraded to small fragments and therefore the nucleoid region of the bacterium becomes dispersed. Some phage mRNA is formed immediately after the infection. The number of phage DNA increases after a short delay. Virion specific proteins appear somewhat later, followed by appearance of organized capsid precursors and leading to the formation of mature infectious capsids. Immediate early phage genes are transcribed using the present bacterial RNA polymerase. For the foremost part these genes code for nucleases that break down host DNA and for enzymes that alter the bacterial RNA polymerase so it will preferentially transcribe delayed early phage genes.

Delayed early genes code for phage enzymes which produce unique phage DNA constituent like 5-hydroxy methyl cytosines which glycosylate these nucleotides or which destroy

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precursors of cytosine deoxynucleotide or which destroy precursors of cytosine deoxynucleotide so no bacterial cytosine are incorporated into phage DNA. These alterations enable the phage to survive because bacterial restriction enzymes are unable to degrade phage DNA modified by the substitution of 5-hydroxy methyl cytosine for cytosine and by glycosylation of this substituted base. Further a phage nuclease will destroy any DNA that has unsubstituted cytosine. Delayed early genes also code for polymerases and ligases that play specific role in phage DNA replication and recombination and for a second altered RNA polymerase that may transcribe the late genes.

Step 4 - ASSEMBLY AND RELEASE: Only after the synthesis of both structural and macromolecule is well under way do the phage components begin to assemble into mature phage. About 25 mins after initial infection, around 200 new bacteriophages assemble and therefore the bacterial cell bursts releasing the new phage to infect other bacteria and start the cycle once again.

3.3 LYTIC AND LYSOGENIC MODES OF ACTION [3,6]

Following a bacterial infection either a lytic or a lysogenic cycle can occur:

3.3.1 LYTIC PATHWAY:

It is promoted by a lytic bacteriophage which is virulent bacteriophage. The bacterial metabolic content is confiscated by the latest bacteriophage assembly. This causes replication of the bacteriophage genome inside the bacterial cytoplasm and also synthesis of viral proteins.

3.3.2 LYSOGENIC CYCLE:

The bacteria is more virulent and immune to some of the bacteriophages. The bacterial cell has an integrated prophage in its genome that is in a latent state even during cell division. Thus allow bacteriophage cycle for replication through the lytic pathway with further release of new virions.

In either of the two cycles viz., lytic and lysogenic cycles, after the synthesis of bacteriophage proteins and enzymes the assembly and formation of latest virions occurs. For the release of these newly formed virions, there has to be disruption of the cell wall by holin by perforating the membrane and facilitating translocation of lysins on peptidoglycan layer.

IV. APPLICATIONS IN SYSTEMIC INFECTIONS

4.1 RESPIRATORY AND PULMONARY INFECTIONS [3,7,8]

A satisfactory result seen in patient with P. aeruginosa septicaemia P. aeruginosa aortic graft infection after successful bacteriophage therapy. An anti-staphylococcal phage preparation showed around 70% and 55% rate of successful reports in the treatment of staphylococcal infection and staphylococcal sepsis respectively.

Phage therapy can be effective in patients with chronic multi drug-resistant disorders and infections. A cystic fibrosis patient with chronic multidrug-resistant develop P. aeruginosa pulmonary infection. It is a need of the hour to have in depth research with respect to lung model development for phage therapy and clinical trials for effective treatment for cystic fibrosis patients.

4.2 GASTROINTESTINAL INFECTIONS [3]

Human gut is densely populated by varied virus families and the most widespread are Siphoviridae, Myoviridae and Podoviridae.

In the past, bacteriophage has given good results against cholera infections in India and Eastern Europe, also the reports on prophylactic bacteriophage treatment against dysentery in Soviet soldiers revealed a 10-fold lower incidence of dysentery when compared to the soldiers who did not receive the phage therapy. Bacteriophage were demonstrated to be effective in combating Shiga-toxin producing E. coli O104:H4 that had caused hemolytic uremic syndrome and bloody diarrhoea in Germany in the year 2011. This shows that bacteriophage shows a superior alternative for treating infections caused by antibiotic-resistant pathogens.

4.3 SKIN INFECTIONS [3,9]

P.s aeruginosa is a well-known etiological agent in wound infections with known multiple antibiotic resistant strains created posing problems in the treatment. Skin infections caused by Mycobacterium marinum, Mycobacterium szulgai or Treponemapertenue also create difficulties in the treatment of dreadful skin infections by these bacteria and therefore developing adequate approaches for targetting bacteriophage delivery into mammalian cells is more challenging. Mycobacteriophage D29 is found efficient in a therapy against M. ulcerans.

4.4 ACNE TREATMENT [3,10]

P. acne that elicits inflammatory response that give rise to acne lesions are topically treated by antimicrobial chemicals, oral antibiotics or retinoids. These topical agents though tolerated well, in some patients show dermal irritation, scaling or itching. Oral antibiotics prescribed for up to six months developed antibiotic resistant strains of P. acne strains. They may also show other adverse reactions that include dyslipidaemia,

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altered blood glucose levels, eye and skin disorders and mood disorders. Lytic bacteriophage (phage) acts as a substitute to such antibiotic therapy. Bacteriophages are highly specific that lyse only the bacterial hosts. They are medically safe, bring about minimal disruptions to the autogenous microbial community. They also lyse antibiotic resistant strains. 4.5 4.5 DENTAL INFECTIONS [3,11]

Bacteriophage control bacterial growth which otherwise affects the balance between health and disease in the oral cavity. Bacteria present in the oral cavity colonize and grow as biofilms on various oral surfaces such as the gingival epithelium, the teeth and the oral mucosa that lead formation of caries, xerostomia, periodontal disease and dental pulp infections. Adhesion of bacteria in the oral cavity areas cause biofilms which is an irreversible effect. If the bacteria are not accessible to chlorhexidine and antibiotics they escape the oral immune system, cause chronic oral diseases. This inaccessibility of antibacterials and antibiotics against such resistant bacterial population lead to dental infections which are difficult to treat. Bacteriophage therapy can be a better option to combat dental infections.

4.6 DENTAL CARIES [3]

Streptococcus mutans cause dental caries by forming dental plaque and further erosion of the tooth. Use of bacteriophage have been studied to remove S. mutans from dental plaque and dental caries. A phage specific for S. mutans was isolated from a saliva sample that belong to the Siphoviridae family. This phage showed a very strong lytic effect against S. mutans and lesser lysogenic activity. The phage activity against S. mutans in the biofilms was observed after 48 h. At doses of 105-109 PFU/well, the bacteriophage completely inhibited metabolic activity of the biofilm.

4.7 ENDODONTIC INFECTIONS [3]

Endodontic disease is due to formation of biofilm related infection in the dental pulp. The treatment of such condition includes proper cleaning measures in combination with use of topical chemical agents. Here again bacterial resistance response to the etiologic agents has been reported. Therefore isolation of bacteriophage have been carried out specific for Enterococcus faecalis, which is responsible for endodontic infections and antimicrobial resistance.

Bacteriophage therapy lacks more sophisticated, modern, precisely controlled and double-blind clinical trials. Only a few bacteriophage therapy clinical trials have been conducted so far. The most significant clinical trials of bacteriophage therapy performed so far are presented in Table 1:

Table 1: Overview of clinical trials conducted on bacteriophage therapy

Clinical trial	Trial phase	Target Bacterium	Phage(s) used	Observations
Venous leg ulcers	I	Escherichia coli; Staphylococcus aureus; Pseudomonas aeruginosa	WPP-201	No adverse effects observed
Chronic ear infections	I/II	Pseudomonas aeruginosa	Bio phage-PA	Significant improvement observed
Diarrhoea	I/II	Escherichia coli	T4 coli	No adverse effects observed

V. DRUG DELIVERY SYSTEMS

5.1 INHALATION DELIVERY OF BACTERIOPHAGE [12]

Bacteriophage capable of treating pulmonary infections have been evaluated in vitro and in animal models and showed satisfactory results where the phage was administered through nebulisation. In respiratory tract infections, the bacteriophage can be administered locally to the lung tissues that allow higher concentrations to be accumulated at the site of infection. Thus avoids unnecessary distribution of them to other sites. This improves the activity and lowers the chance of any adverse effects.

5.2 AEROSOLS [3,13]

Gram negative bacteria Burkholderiacepacia complex significantly affects cystic fibrosis patients. In vitro testing of lyophilized bacteriophages against Burkholderiacepacia complex and P. aeruginosa showed promising result and could be dispersed in an aerosolised form. Lyophilization thus facilitates

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delivery of bacteriophage as inhalation. Another such bacteriophage administered intra peritoneally and as an aerosol gave satisfactory outcome. Aerosolied form was much more effective in treating pneumonia than intraperitoneal route.

5.3 INTESTI PHAGE [3]

Intesti Phage preparations are bacteriophage used for prophylaxis and treatment of intestinal infections due to Shigella, Salmonella, Proteus, Staphylococcus, E. coli and Pseudomonas. An important challenge in bacteriophage therapy for gastrointestinal infections is the presence of gastric acid that can destroy the phage administered. It has been observed that use of polymer microencapsulation of bacteriophage has been very protective and effective in this regard. Administration of bacteriophage with probiotics in the treatment of dysbiotic disorders of the human gut gave promising results.

5.4 BACTERIOPHAGE ENCAPSULATION [14]

A polymer or lipid may be used to coat an existing structure containing the phage to protect it from physical and chemical stresses and improve long term stability. There are several processes that may be used for stabilising, immobilising and encapsulating phage. The most widely used methods are spray drying, spray freeze drying, freeze drying, extrusion dripping methods, emulsion and polymerisation techniques.

5.5 TRANSDERMAL DELIVERY [3,15]

Bacteriophage particles (e.g. Caudovirales), being hydrophilic differ from small and lipophilic drug molecules as they do not cause efficient transdermal absorption. Design and evaluation of a novel hollow polymeric microneedle device for the transdermal delivery of bacteriophage particles has been reported and it delivered viable T4 bacteriophage transdermally both in vitro and in vivo. This transdermal delivery as microneedle device punctures the skin avoiding the stratum corneum to create temporary aqueous transport pathways of micron dimensions thus enhancing permeability and absorption across the skin.

5.6 PHAGE-DERIVED PROTEINS AND ENGINEERED BACTERIOPHAGE [16]

Bacteriophage utilize lysins to degrade the bacterial cell wall. These endolysins act as antibacterials against gram positive bacteria while in the gram negative the outer membrane acts as a shield for the endolysin susceptible peptidoglycan layer. To degrade gram negative bacteria it is essential to disorganize the outer membrane which can be possible by combining lysins with other agents that can destabilize the outer membrane, or by genetic engineering of endolysins. Possible antimicrobial implementation of genetically engineered bacteriophage includes methods so as to enhance antibiotic activity such that it can prevent antibiotic resistance.

5.7 ADVANTAGES OF PHAGE THERAPY [5]

- Bacteriophage are available where needed as they replicate at the site of infection
- No adverse side effects
- Phage resistant bacteria are susceptible to other phage
- Selecting new phage to work in place of a phage-resistant bacteria is a generally a rapid process completed in days or weeks.
- Oral phage administration is generally considered to be safe
- Very specific, usually affect only the targeted bacterial species

5.8 PHAGE THERAPY IN INDIA [17,18]

Vitalis Phage Therapy recently launched in India to eliminate antimicrobial resistance. Vitalis Phage Therapy facilitates phage therapy offered by the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi, Georgia. The treatment involves two ways. The first is 'in-clinic treatment' at the Eliava Phage Therapy Center for diagnostic tests and phage treatment. The second is 'distance treatment' where the samples sent for diagnostic testing to the institute's diagnostic lab, and the clinic issues phage medicines for the patient's treatment. The treatment carried out in India.

Vitalis Phage Therapy is working along with local diagnostic laboratories thus making it easier and more accessible for patients in India.

French scientist Felix d'Herelle Phage discovered Phage in 1917 while the anti-bacterial action of phage was identified in 1896 in the water of rivers Ganga and Yamuna**.** Phage therapy started gaining momentum in the West a decade back, and the clinical trials in advanced stages across Western Europe and the US. Rapid growth of antimicrobial resistance in the West gave a driving force for bacteriophage therapy.

VI. CONCLUSION:

Bacteriophage therapy against increasing antibiotic resistance has shown promising outcomes in certain parts of the world but requires use of more specific, sophisticated, modern randomized double-blind controlled clinical trials. This is so as to prove the safety and efficacy of the bacteriophage therapy. Factors such as like choice of bacteriophage, isolation, purification, storage should be looked into and resolved individually. Regulatory issues with respect to production of bacteriophage preparations are challenging in

phage therapy products. Nevertheless bacteriophage is promising alternative for treatment of bacterial infections against antimicrobial resistance. Other than the natural bacteriophage, the phage-derived endolysins and genetically engineered bacteriophage are also as effective as the antimicrobials. Looking at the safety and specificity factors of bacteriophage and broad spectrum of applications of bacteriophage against systemic infections, they can be considered as a boon for treatment of systemic infections.

CONFLICT OF INTEREST

There is no conflict of interest.

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